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PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Docket No: Q76104

Hiroshi TAKEYAMA, et al.

Appln. No.: 10/611,902

Group Art Unit: 1614

Confirmation No.: 8672

Examiner: Amy A. Lewis

Filed: July 3, 2003

For: ANTITUMOR AGENT

DECLARATION UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Hiroshi Takeyama, hereby declare and state:

THAT I am one of the inventors of the invention disclosed and claimed in the above-identified application.

THAT I am a citizen of Japan;

THAT I have received the degree of Medical Doctor (M.D.) in 1983 from National Government of Japan;

THAT I have been employed by Jikei University, School of Medicine since 1983, where I hold a position as assistant professor, with responsibility for Department of Surgery;

The following experiments were performed by me or under my direct supervision.

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U.S. Appln. No.: 10611,902

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CERTIFIED EXPERIMENT RESULT I

In vitro Antitumor Effect for Stomach Cancer Cell line (STKM) by benzyl alcohol (BA)

Materials and Methods

To 1×10^6 cells of a stomach cancer cell line (STKM), BA was added in proportional concentrations, such as 931, 465.5, 232.8, 116.4, 58.2, and 29.1 $\mu\text{g/ml}$, respectively, followed by incubating for 48 hrs at 37°C . As a control, the same amount of physiological salt solution was added in place of BA. Cells under each condition were observed by invert microscope, followed by determination by Colorimetric assay of change in adhesive property and cell death.

Colorimetric assay

100% glutaraldehyde was added to one fourth (250 μl) of each cell sample. The cell samples were then incubated for 15 minutes at room temperature without shaking, followed by washing. Then 250 μl of 0.05% ethylene blue in PBS was added. Then 250 μl of 0.33 N HCl was added, followed by incubation for 15 minutes without shaking. Finally, the OD (595 nm) was measured.

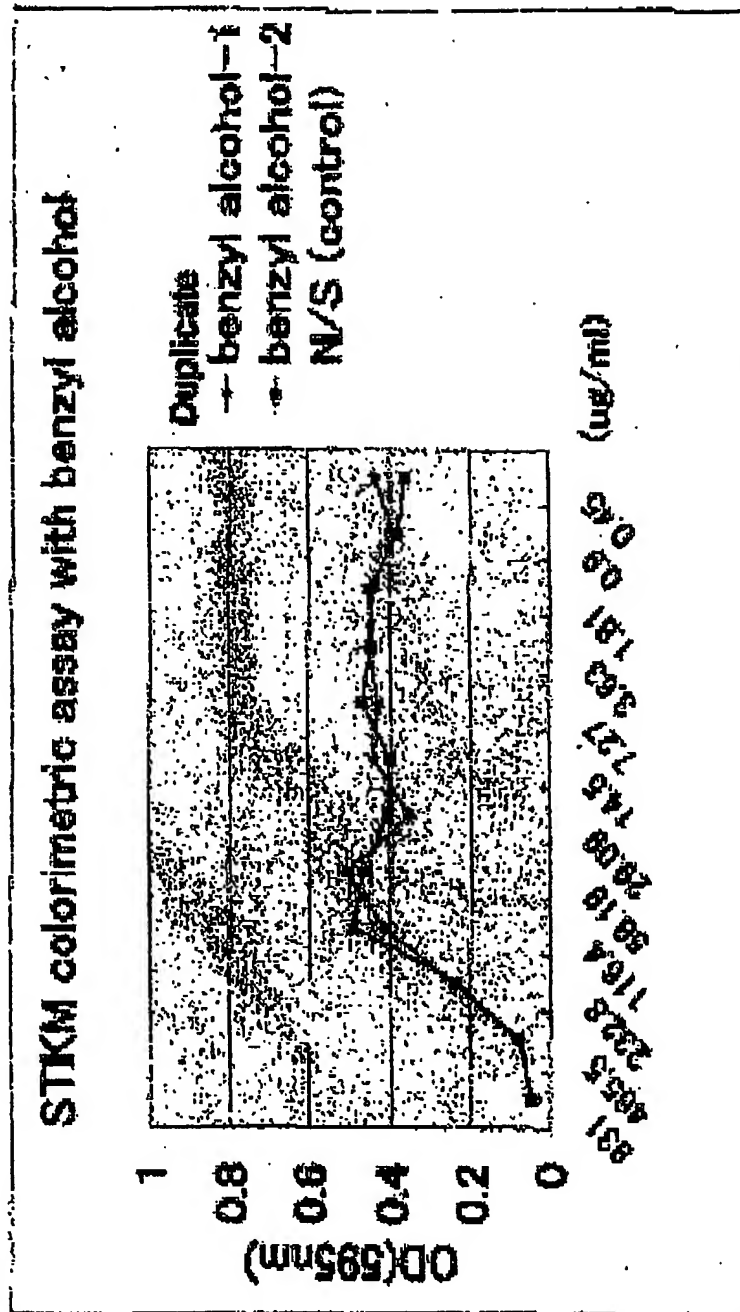
Results

The adhesive property of the cells was proportionally decreased and the death of the cells was proportionally increased as far as BA was added in proportional concentrations, such as 931, 465.5, 232.8, and 116.4 $\mu\text{g/ml}$, respectively, while a change in adhesive property and cell death was not observed in concentrations of 50 $\mu\text{g/ml}$ (Fig. 1).

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Figure.1



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CERTIFIED EXPERIMENT RESULT 2

In vitro Antitumor Effect for Stomach Cancer Cell line (STKM) by combination use of benzyl alcohol plus vitamin C (BA+C)

Materials and Methods

To 1×10^6 cells of a stomach cancer cell line (STKM), BA+C was added in proportional concentration, such as 10,000(BA)+100,000(C), 5,000+50,000, 2,500+25,000, 1,250+12,500, 625+6,250, 78.1+781, 39.1+391, 19.5+195, 9.76+97.6 and 4.9+49 $\mu\text{g/ml}$, respectively, followed by incubating for 48 hrs at 37°C . As a control, the same amount of physiological salt solution was added in place of BA. Cell samples under each condition were observed by invert microscope, followed by determination by Colorimetric assay of change in adhesive property and cell death.

Colorimetric assay

100% glutaraldehyde was added to one fourth (250 μl) of each cell sample. The cell samples were then incubated for 15 minutes at room temperature without shaking, followed by washing. Then 250 μl of 0.05% ethylene blue in PBS was added. Then 250 μl of 0.33 N HCl was added, followed by incubation for 15 minutes without shaking. Finally, the OD (595 nm) was measured.

Result

The adhesive property of the cells was proportionally decreased and cell death was proportionally increased as far as BA + C was added in proportional concentrations, such as 10,000(BA)+100,000(C), 5,000+50,000, 2,500+25,000, 1,250+12,500, 625+6,250, 78.1+781, 39.1+391 $\mu\text{g/ml}$ concentration, respectively, while a change in adhesive property and cell death was not observed in concentrations of 19.5(BA)+195(C), 9.76+97.6 and 4.9+49 $\mu\text{g/ml}$, respectively.

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I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 03.30. 2006.

Hiroshi Takeyama
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